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THE first recorded observations on a copper-deficiency condition in livestock were made by Neal in Florida in 1931. Shortly afterwards in Holland Sjollema showed that a wasting or pining disease in cattle, known in that country as liksucht, could be cured by the administration of copper sulphate. Within a few years extensive investigations were in progress in Australia and New Zealand, and it was found that copper therapy effected spectacular improvement in condition among sheep and cattle suffering from a debilitating disease common in the coastal areas. In England in 1939 investigations showed that the disease known as " swayback " in lambs could be prevented by the administration of copper to the pregnant ewe. Soon afterwards, Ferguson and co-workers (1) successfully treated the scouring disorder in cattle on the teart pastures of Somerset by the feeding of supplemental food fortified with copper sulphate. Following the pioneer work of Allcroft, Russell and Jamieson on hypocupraemia in cattle in England and Scotland, regional surveys during the succeeding years showed that pining disorders in grazing cattle in widespread areas of Great Britain were amenable to treatment by oral administration of copper salts.

The work reported in this paper had its origin in certain observations made by Sheehy and O'Donovan during the course of investigations on aphosphorosis in cattle in Offaly in the years 1945–1948 (2). They noticed that on some farms in the extensive phosphate deficient areas which they found in that county, certain cattle failed to respond to the feeding of supplementary phosphate. These animals remained in a very unthrifty, backward condition, and suffered from an acute scour of a distinctive dark green colour and of a frothy consistency. Growing stock remained stunted, and there were many cases among them of depigmentation of hair and coat. Remedial measures for worm infestation in most cases failed to effect any improvement. Although there had been no recorded cases in Ireland of hypocupraemia, certain of the symptoms suggested copper or cobalt deficiency, and it was decided to try the effect of drenching with solutions of salts of these elements. It was soon observed that, at least in the worst cases, a single dose of 5 g. of copper sulphate brought about an immediate and spectacular response. Arrangements were made later to extend the scope of the investigation and to collect and analyse samples of cattle blood and of herbage taken from the areas which the preliminary investigation had shown to be the worst affected.

* Since date of reading some additional analytical results have been included.

Experimental.

Analysis of Blood Samples.

With a view to restricting the investigation to uncomplicated cases of the disorder, it was decided to examine carefully the general condition of the animals on an affected farm before taking blood and herbage samples. If the preliminary investigation revealed that the scour or unthriftiness could be accounted for by worm infestation, or if the herd displayed advanced symptoms of aphosphorosis, the farm was considered unsuitable for further investigation. Among the animals on a particular farm no attention was given to those which had been brought on to the farm from another district during the previous 12 months.

The blood samples were drawn in the usual way from the jugular vein into 1-oz. bottles containing 55–60 mg. of potassium oxalate. The initial stages of the copper estimation were usually performed on the same evening. The method used was that of Eden and Green (3) for the micro-determination of copper in biological materials, with modifications suggested by Clarke, Cunningham, and Perrin (4). A feature of the distribution of the disorder was that affected and unaffected farms adjoined each other in the various districts. It was considered desirable as a control measure to have numerous blood samples drawn from normal animals on the unaffected farms. Table 1 gives the results of the analysis of these samples.

Table 1.—Blood Copper Levels of Animals on Farms where Symptoms were Absent.

Farm No.	No. of animals tested	Blood Copper (mg./100ml.)
1	6	·10, ·11, ·09, ·10, ·10, ·10,
2	2	·10, ·09.
3	9	·10, ·09, ·10, ·10, ·12, ·07, ·11, ·09, ·10.
4	8	·11, ·11, ·09, ·09, ·10, ·09, ·09, ·07.
5	7	·09, ·07, ·09, ·06, ·10, ·07, ·13.
6	6	·11, ·07, ·10, ·10, ·12, ·11.
7	9	·08, ·09, ·10, ·07, ·08, ·09, ·08, ·11, ·08.
8	7	·10, ·10, ·10, ·08, ·09, ·10, ·10,
9	4	·07, ·11, ·09, ·08.
10	6	·08, ·07, ·07, ·09, ·11, ·10.
11	13	·10, ·07, ·07, ·10, ·06, ·07, ·09, ·07, ·08, ·09, ·12, ·09, ·09.
12	2	·11, ·13.
13	4	·08, ·05, ·11, ·10.
14	4	·11, ·09, ·10, ·09.
	87	

It is usually considered that for cattle, a blood copper level of ·07 mg. per 100 ml. or higher may be considered normal, that ·06 and probably ·05 are marginal, and that ·04 and less may be reckoned as abnormally low. Accepting this arbitrary interpretation of the analytical figures, it will be seen that of the blood samples from 87 cattle on these 14 unaffected farms, 84 were normal, 3 marginal and none low.

The routine procedure adopted in the case of affected farms was, firstly, to ascertain whether the disorder could be due to worm infestation or could be the result of aphosphorosis. If it was considered that these conditions were absent, blood samples were taken from all the cattle or calves showing definite symptoms of pining or scouring, however slight. Dosing with copper sulphate was postponed until these first samples had been analysed. On the occasion of the second visit, blood samples were again drawn, and the copper sulphate then administered orally. After an interval of 2 to 3 weeks further blood samples were taken, and, if these did not reveal copper levels within the normal range, further drenches were given and after an interval further blood samples taken. In some cases three or four drenches were necessary and the same number of blood samples were drawn for analysis. After some preliminary tests it was decided that, as a routine experimental procedure, the drench would consist of 5 g. of copper sulphate of Analar quality. This amount was weighed out in the laboratory beforehand and dissolved in about half a pint of water on the farm.

Table 2 gives the result of these assays. In preparing the table, the highest figure obtained for blood copper for each animal in both the pre-dosing and post-dosing period has been inserted. The total number of blood samples drawn from the cattle on affected farms and analysed for copper was 210.

Table 2.—Blood Copper Levels of Animals showing Symptoms (mg./100 ml.).

Farm	No. of Animals	Before Dosing	No. of Animals	After Dosing
A	1	·05.	1	·09.
B	4	·04, ·03, ·03, ·03.	3	·05, —, ·08, ·07.
D	2	·04, ·04.	2	·07, ·06.
G	5	·02, ·04, ·03, ·02, ·03.	5	·06, ·04, ·06, ·05, ·07.
H	6	·04, ·06, ·05, ·03, ·04, ·04.	6	·09, ·08, ·08, ·09, ·08, ·09.
I	5	·04, ·04, ·02, ·03, ·07.	4	·07, ·08, ·05, ·05, —.
J	6	·04, ·03, ·02, ·04, ·05, ·03.	4	·06, ·06, ·07, —, —, ·05.
K	5	·04, ·05, ·04, ·04, ·04.	5	·08, ·06, ·05, ·03, ·02.
L	5	·03, ·03, ·03, ·03, ·04.	5	·07, ·07, ·08, ·07, ·08.
M	1	·03.	1	·07.
N	1	·04.	1	·05.
O	6	·03, ·02, ·03, ·04, ·02, ·03.	6	·08, ·07, ·07, ·04, ·05, ·06.

The figures show that the dosing with copper sulphate consistently brought about a remarkable increase in blood copper levels. In many cases a single dose of 5 g. had this effect but, as already mentioned, in some cases repeated drenching was necessary. The figures in Table 2 show that ultimately only an occasional animal failed to respond, due probably to insufficient intake of copper sulphate.

A summary of Table 2 can be prepared using the accepted ranges mentioned above for normal, marginal and low blood copper levels.

Summary of Table 2.

No. of Animals			Blood Copper Levels (mg./100ml.)		
			Normal	Marginal	Low
Before Dosing	...	... 47	1	5	41
After Dosing	...	... 43	24	15	4

The 47 grazing animals under test comprised 42 cows, 4 bullocks and 1 calf. The 4 animals missing in the post-dosing period had been sold.

The summarised results confirm that the general effect of dosing with copper sulphate was to bring about a marked increase in blood copper concentration. A more spectacular result of the administration of copper sulphate was the immediate cessation of scouring in the great majority of cases studied. Occasionally a second or third dose was required to achieve this. Only very occasionally did scouring recur during the ensuing months of the grazing season. It was not found possible to record weights or to measure growth rate in the animals under test, but mere inspection revealed that with few exceptions there was a noticeable improvement in condition following the cessation of scouring. Ample confirmation of this observation was afforded by reports from farmers owning these cattle, and by the interest shown in obtaining the remedy for the unthrifty condition.

Analysis of Herbage Samples.

In sampling the pastures an effort was made to collect samples as representative as possible of the area being grazed. Nevertheless those spots where growth was unusually rank or where weeds predominated were in general not sampled. Neither was any material taken from hedgerows or field boundaries. Consequently, it is possible that the herbage samples analysed were not truly representative of the grasses and weeds actually consumed by the animals. Moreover, in many cases the pasture samples were not taken until some weeks after the blood had been assayed for copper. All herbage samples were air-dried, ground in a laboratory mill, and analysed for total copper and molybdenum some months later. The copper was

again determined by the method of Eden and Green as used for the assay of the blood samples. For the determination of molybdenum in the pasture samples the technique worked out by Perrin (5) was used.

Table 3 shows the figures obtained for copper in the pasture samples. The insertion of more than one figure for a particular farm indicates that certain fields were analysed as separate units.

Table 3.—Copper Content of Pasture Herbage (parts per million on dry matter basis).

Unaffected Farms			Affected Farms			
Farm	Summer 1952	Autumn 1952	Farm	Autumn 1951	Summer 1952	Autumn 1952
1	8·8	8·8	A	11·0	10·0	10·0
2	9·9	9·8	B	10·5	15·0	8·8
3	9·9	7·7	D	8·8	9·9	6·6
4	7·6	—	G	10·1	13·2	9·9
5	6·6	5·5	H	11·1	10·0	8·7
6	9·8	7·7	I	9·9	10·0	7·7
7	7·7	10·0	J	7·7, 9·8, 9·3	14·3	8·8
8	10·0	—	K	12·1, 8·8	7·8	—
9	7·7	9·3	L	9·7, 9·3, 7·7	8·9	7·8
10	12·6	10·0	M	8·8	10·0	9·8
			N	12·1, 14·3	14·8	12·0
			O	9·3, 9·3, 6·6	11·0	11·0
Means	9·1	8·6		9·8	11·2	9·2

The identification numbers and letters for the farms used in Table 3 correspond to those of Tables 1 and 2, respectively. The copper figures range from 5·5 to 12·6 for the unaffected pastures on which there was no indication of pining or scouring, and from 6·6 to 15·0 for the affected farms. There is no suggestion of any significant difference between the two groups of figures, the mean of the 18 determinations on the normal farms being 8·9 and of the 43 determinations on the affected farms being 10·1. The range of the figures for both types of farm is in conformity with that generally accepted as being normal for the copper content of non-deficient pastures.

Table 4 shows the figures obtained on analysis of the pastures for total molybdenum. Again, as indicated by the appearance of more than one figure in a column, certain fields on some farms were treated as separate units.

Table 4.—Molybdenum Content of Pasture Herbage (Parts per million on dry matter basis).

Unaffected Farms			Affected Farms	
Farm	Summer, 1952	Autumn, 1952	Farm	Autumn, 1951
1	2·2	1·1	A	15·4
2	3·3	2·2	B	22·0
3	2·2	2·2	D	11·0
4	2·2	—	G	16·6, 17·6
5	3·3	3·3	H	14·8
6	2·2	2·7	I	23·1
7	7·7	7·7	J	12·6, 17·6, 9·9
8	2·7	—	K	19·8, 32·7
9	3·3	3·8	L	14·3, 8·8, 11·0
10	2·2	1·1	M	19·2
			N	14·3, 18·7
			O	10·4, 8·8, 7·7
Means	3·1	3·0		15·5

The molybdenum figures in contrast to those for copper show a pronounced difference between the herbage from the unaffected and from the affected farms. The range in the former case is from 1·1 to 3·8 (ignoring one exceptionally high figure of 7·7) and in the latter from 7·7 to 32·7. The mean value of the 18 determinations on the normal farms was 3·0 p.p.m. of molybdenum and of the 21 determinations on those farms where the disorder was present 15·5 p.p.m.

Attention is drawn to the fact that the herbage samples from the unaffected farms were not taken until the summer and autumn of 1952, whereas those from the affected farms were collected in the autumn of 1951, during the period when many of the blood samples of Table 2 were being obtained. In view of the striking differences between the two sets of figures for pasture molybdenum, it is considered most unlikely that the result would have been otherwise had all pastures been analysed in the same season.

DISCUSSION.

Certain conclusions may be drawn from the results obtained in this preliminary and restricted survey in Offaly. Firstly, the disordered condition is accompanied by a pronounced fall in the level of blood copper. Secondly, oral administration of 5 g. of copper sulphate, repeated if necessary at intervals of 2 to 3 weeks, relieves the

condition and restores the blood copper to normal levels. Thirdly, there is no deficency of copper in the pasture herbage. Fourthly, the molybdenum content of the Offaly pastures in which the diseased condition is found, while definitely below the range of molybdenum found in the teart pastures of Britain, is significantly higher than that of the pastures in the same district on which cattle remain in normal health. It may be of interest to compare these observations and findings with those published in the literature of the subject by investigators dealing with the same problem in other regions.

A striking feature revealed by any such survey is the remarkable absence of uniformity in symptoms and apparent causes of the diseased condition in different regions and sometimes even among different farms in the same district. In fact, the only factor common to all cases is the ease with which the condition is remedied by the administration of copper. Thus, the persistent scouring as observed in Offaly has been described as a symptom in many but not in all reported cases. For example, diarrhoea was absent in the Cambridgeshire cases of hypocupraemia and pining in calves and heifers described by Blackmore and Venn (6). Pica or depraved appetite manifests itself as a symptom in some areas of Britain, but not in others; in Offaly pica is associated with aphosphorosis in cattle, but not usually with hypocupraemic disorders. Loss of pigment in the hair is a fairly general manifestation, especially among affected calves. In Caithness, Jamieson and Allcroft (7) report that calves are affected so adversely that there is considerable mortality among them, whereas in the same area adult cattle are not affected. Moreover, the same authors have drawn attention to a breed difference in susceptibility in the same area, Aberdeen Angus calves being more affected than Shorthorns.

The indications of a diseased condition universally present are pronounced unthriftiness, some degree of emaciation and stunted growth. In dairy cows, anoestrus is fairly common and this was observed in Offaly. Analysis of samples of blood and liver from affected animals nearly always reveals the existence of a low copper status at least in advanced cases of the disorder. The blood of cattle normally contains about ·1 mg. of copper per 100 ml., and it is generally considered that a content of less than ·07 mg. per 100 ml. is abnormal. Conversely, however, it is now well recognised that low blood copper is not in itself a disease, and investigators have frequently found apparently healthy and thrifty animals with blood copper levels as low as ·04 mg. per 100 ml. There are many references to the fact that a fall in blood copper concentration may occur in individual animals as a secondary feature in certain organic diseases. It is now believed that liver copper reserves are a truer indication of health or disease in this regard. Since the perfection of the methods of biopsy, whereby samples of liver for analysis can be taken from the living animal, the routine assay of liver for copper reserves has become an important diagnostic technique.

A review of the nutrient status of the pastures throughout the world on which the condition of hypocupraemia develops reveals a diversity of conditions which,

so far, has prevented any rational diagnosis of the fundamental causes of the disease. In New Zealand and Australia the pastures on which the pining diseases occur usually contain less than normal amounts of copper, assuming that the normal range is from 5 to 15 p.p.m. In Britain, however, there has been no consistent finding of low copper values for the pastures grazed by the affected animals, even in those cases where blood copper values were as low as ·02 mg./100 ml. Hence the conclusion by many research workers that the peat scours of New Zealand are either a simple hypocuprosis due to deficiency of copper in the pastures or else are caused by a small excess of mólybdenum (2–11 p.p.m.) superimposed on a moderate deficiency of copper. In England, and apparently in Offaly, all cases reported are of the type known as a " conditioned " copper deficiency, where, although the dietary copper intake is normal, some other factor or factors in the pasture herbage seriously interferes with the assimilation, metabolism or storage of copper.

The discovery that the teart pastures of Somerset were normal in copper content but exceptionally high in molybdenum, and the fact that the scouring could be cured by the administration of copper sulphate, led to the view that molybdenum was the factor responsible for most cases of hypocupraemia in cattle grazing pastures of normal copper content. It is now recognised, however, that the molybdenum content of the Somerset soil is exceptionally high due to a rare geological formation whereby outcrops of molybdeniferous rocks occur in that area. In addition, the soil is alkaline, and in recent years it has been well established that certain pasture plants will absorb molybdenum very readily from alkaline soils. Many investigators, moreover, have stressed the difference between the severe scouring on true teart pastures and the pining disorder in cattle accompanied by hypocupraemia occurring elsewhere.

Green (8) draws attention to the fact that hypocupraemia with or without scouring has been observed outside the teart areas on pastures where the molybdenum content is as low as 3 p.p.m. Considering also that severe scouring can be readily brought about by dosing stalled cattle with molybdenum salts, Green and others have expressed the view that the true teart disorder is a case of simple molybdenosis, and that other factors are involved in the vast majority of cases observed outside the teart areas. Many investigators hold the view that on pastures of intermediate molybdenum content the element may act as a predisposing cause, either because copper intake is low or because there may also be present another factor interfering with copper metabolism.

Attempts to identify a second interfering factor have not, so far, met with much success. In the investigations carried out by Ferguson *et al.* (1) on the teart pastures of Somerset it was shown that teart hay on storing for one year in a stack no longer caused scouring when fed to cattle. Offaly farmers are aware also that scouring can be cured by feeding hay, even when it has been saved from the pastures grazed by the affected stock. A most significant result in this connection has been published by Dick *et al.* (9). They fed groups of sheep on various mixtures of lucerne hay and of oaten hay. The total intake of copper and molybdenum for all groups was

adjusted to 15 mg. and 10·7 mg., respectively, per sheep daily. It was demonstrated that oaten hay had the effect of enormously increasing the level of molybdenum in the blood. Moreover, while in previous tests this level of dietary molybdenum had greatly reduced the storage of copper in the liver, it was now shown that oaten hay had the effect of modifying this action of molybdenum, thereby enabling the liver to store considerable quantities of copper during the feeding period of 6 months. In support of the view that the "conditioning" factor in cases of hypocuprosis is not molybdenum, Green and Allcroft (10) refer to the finding that systematic administration of molybdenum on affected farms does not accentuate the diseased condition. They point out also that, in their experience, no significant correlation exists between the incidence of the condition in cattle and the level of molybdenum in the pastures.

Barshod (11) has demonstrated that cattle grazing on a pasture containing as much as 100 p.p.m. of molybdenum in the dry matter of the herbage remained in normal health if dry roughage were given as supplemental food. It seems fairly certain, therefore, that the molybdenum content of the pastures is not the only causative factor involved in the disorder.

It is, however, unwarrantable to rule out molybdenum as a causative factor in the condition described in the Offaly cattle because of the fact that the molybdenum content of the pastures did not reach the lower end of the teart scale, which is usually considered to range from about 20 to 100 p.p.m. It is impossible to consider the differences between the molybdenum figures for the herbage of the affected and unaffected farms shown in Table 4 as anything but highly significant. Moreover, in the report already referred to, Barshod and co-workers consider 20 p.p.m. too high as a safe upper limit for the molybdenum content of pastures. They also draw attention to the now well-known fact that a relatively small amount of molybdenum in young luscious plants is more dangerous than a much larger amount in mature herbage. Lewis (12) observed that on the Somerset teart pastures, clovers and Yorkshire Fog were outstanding in their ability to absorb molybdenum from the soil. Barshod (11) has also shown that on the same pasture fields different varieties of grasses and weeds can absorb widely varying amounts of molybdenum, especially under alkaline soil conditions, even when the soil has a low molybdenum content. In one case the range of molybdenum in the various pasture plants extended from 1 to 22 p.p.m. even though the soil contained only a trace of total molybdenum. With a soil rich in molybdenum he found the range to be from 2 to 200 p.p.m. depending on species.

Many workers have drawn attention to the fact that, especially where pastures contain a high proportion of legumes, heavy liming of acid soils high in molybdenum may result in a greatly increased uptake of molybdenum by the pasture herbage.

Such observations suggest that at least on certain types of pasture a herbage sample, although representative of the area, may not be strictly representative of the grasses and weeds, which a particular animal may be consuming, and that con-

sequently a very misleading impression of the molybdenum intake of the grazing animal may be formed. Had it been possible to give more attention to identifying the grasses plants and weeds, actually being consumed each day by the grazing cattle in Offaly an even higher intake of molybdenum might have been revealed. It was observed on numerous occasions in Offaly that even on affected farms certain fields in the experience of the owner were known to be much worse than others in the matter of causing scouring and unthriftiness. It is felt that in any extension of this survey which may be undertaken particular attention will need to be given to the question of drawing herbage samples truly representative of the species being consumed by the grazing animal.

Summary and Conclusions.

An investigation has been conducted in the county of Offaly into cases of unthriftiness accompanied by pronounced scouring in grazing cattle. Animals believed to be suffering from worm infestation or from the condition of aphosphorosis were rejected as experimental material.

Analyses of samples of blood from 47 badly affected animals showed that in nearly all cases the level of copper was abnormally low—the concentration being ·04 mg. or less per 100 ml. in 41 of these cases. As a control measure, samples were drawn from 87 unaffected cattle in the same region and 84 of these samples contained ·07 mg. or more of copper per 100 ml.

A drench of 5 g. of copper sulphate gave immediate relief from scouring in the majority of cases, and also resulted in raising the copper concentration of the blood to normal levels. Occasionally, further doses of copper sulphate were necessary, and ultimately only a few animals failed to improve, due in all probability to insufficient intake of copper sulphate.

Analyses of herbage samples from affected and unaffected farms showed that the copper content of both was normal. The range was from 5·5 to 15·0 p.p.m. on a dry matter basis there being no significant difference between the copper figures for the various pastures examined.

Herbage samples to the number of 30 were also analysed for molybdenum and it was found that a significant difference existed between the figures for those pastures on which the pining disorder never occurred and those on which it was prevalent. In the former case the range of molybdenum was from 1·1 to 3·8 p.p.m. (excluding a single higher figure of 7·7) and in the case of the affected pastures the molybdenum range was from 7·7 to 32·7 p.p.m. with a mean value of 15·5 p.p.m.

While accepting the view of other investigators that another factor is also operating in such cases of pining among grazing cattle, it is concluded that in the cases investigated in Offaly a moderate excess of molybdenum in the herbage is the chief causative factor.

The view is expressed that closer attention to the particular pasture plants being consumed by grazing animals and to their molybdenum content might yield more consistent findings concerning the role of molybdenum as a predisposing cause of the disorder under review.

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